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Spectrofluorimetric analysis of gemifloxacin mesylate in pharmaceutical formulations

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ABSTRACT: A simple, rapid and highly sensitive spectrofluorimetric method was developed for determination of gemifloxacin mesylate (GFX) in tablets. The method is based on measuring the native fluorescence of GFX in isopropanol at 400 nm after excitation at 272 nm. The fluorescence–concentration plot was rectilinear over the range of 0.01–0.50 μg/mL with a lower detection limit of 1.19 ng/mL and quantification limit of 3.6 ng/mL. The method was fully validated and successfully applied to the determination of GFX tablets with an average percentage recovery of 99.65 ± 0.532. The method was extended to the stability study of GFX. The drug was exposed to acidic, alkaline, oxidative and photolytic degradation according to International Conference on Harmonization guidelines. The rate of GFX degradation was found at its highest in acidic conditions, and in its lowest in the neutral one. However, it was stable under dry heat and photolytic degradation conditions. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: gemifloxacin mesylate; pharmaceutical formulations, spectrofluorimetry

Introduction

Gemifloxacin (GFX) (Fig. 1) is a fourth generation synthetic broad-spectrum fluorinated quinolone antibacterial agent for oral administration. It is present in two forms; either as free GFX base or as GFX mesylate salt. GFX has a broad-spectrum activity against both Gram-negative and Gram-positive microorganisms. The main advantages of GFX over the older agents of fluoroquinolones is retaining the excellent activity against Gram-negative bacilli and improving Gram-positive activity (including *Streptococcus pneumoniae* and *Staphylococcus aureus*). Therefore, GFX was approved by the Food and Drug Administration (FDA) in April 2003 for treatment of acute bacterial exacerbation of chronic bronchitis, mild-to-moderate pneumonia multidrug resistant *S. pneumoniae* as well as community-acquired pneumonia (1).

There have been few reported analytical methods for the estimation of GFX in pharmaceutical preparations or biological fluids namely, high-performance liquid chromatography (2–8), high-performance liquid chromatography–tandem mass spectrometry (LC–MS–MS) (9,10), capillary electrophoresis (11,12), voltammetry (13), chemiluminescence (14), spectrophotometry (15–20) and spectrofluorimetry (21).

Among the various methods available for the determination of drugs, spectrofluorimetry continue to be very popular, because of its simplicity, specificity and low cost.

The current study was aimed to develop and validate a simple, rapid and sensitive spectrofluorimetric methodology for the determination of GFX by using its native fluorescence in isopropanol. The proposed method was fully validated according to International Conference on Harmonization (ICH) guidelines, and successfully applied for the determination of the drug tablets and was extended to establish the inherent stability of GFX under different stress conditions such as: alkaline, acidic, oxidative and photolytic conditions. The proposed method has wide linear dynamic range and is more sensitive than reported fluorimetric methods (21).

The stability of the active substance and/or finished product is vital for the effective and safe delivery of the therapeutic values to the patients. This is because the presence of potential degradation products may cause changes in the chemical, pharmacological and/or toxicological properties of the active drug (22,23). Pharmaceuticals are sensitive to environmental variables such as temperature, humidity and light. These factors usually vary during manufacturing, transportation, storage and distribution of the finished product. For these reasons, stability testing of the active substance and the finished product is necessary for providing information about potential degradation products, possible degradation pathways of the drug, compatibility of the drug with the excipients in the finished product and the long-term effects of the environmental factors on the active drug and its finished products. Results of stability testing are important in: developing proper manufacturing processes; selecting proper packaging, storage conditions and the product's shelf life; and determining the expiration date (24,25).

The proposed method can be considered as a simple and fast alternative to the already existing stability indicating HPLC procedure (6).

Experimental

Materials and methods

Apparatus. The fluorescence intensity was measured on a Perkin-Elmer model LS-55 luminescence Spectrometer (UK), equipped

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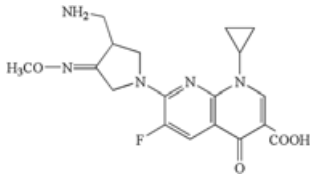

Figure 1. Structure of gemifloxacin.

with a 150 W xenon arc lamp, grating excitation and emission monochromators and a Perkin-Elmer recorder. Slit-widths for excitation and emission monochromators were set at 5.0 and 7.0 nm, respectively. A 1 cm quartz cell was used. pH was measured on a HANNA pH meter (Romania).

Reagents. All reagents were of analytical grade and distilled water was used throughout the work. Pure grade GFX mesylate was kindly supplied from Saudi Pharmaceutical Industries and Medical Appliances Corporation (Al-Qassim Pharmaceutical Plant [SPIMACO] Al-Qassim, Saudi Arabia). The pharmaceutical preparation (Factive® 320 mg/tablet) was provided by (SPIMACO) Saudi Arabia; isopropanol (99.5%) was obtained from BDH (BDH Ltd., Poole, UK).

Sample preparation. A stock solution of GFX mesylate was prepared by dissolving 10 mg of the drug in 100 mL of isopropanol.

General procedure

Construction of the calibration curve. Aliquots of working solutions of GFX mesylate were pipetted in 10 mL calibrated flasks, and diluted to the mark with isopropanol. The GFX mesylate concentration range was 0.01–0.5 μg/mL. The fluorescence was measured at 400 nm using an excitation wavelength of 272 nm against a blank solution. The prepared solutions remained stable for at least 24 h.

Tablet treatment. The total content of 10 tablets was ground to a fine powder. A weighed quantity of the powdered tablets equivalent to 10 mg of GFX mesylate was transferred into a 100 mL volumetric flask, and the flask was sonicated for 30 min. The solution was diluted to volume with distilled water, mixed and filtered. Serial dilutions covering the working concentration range of 0.01–0.5 μg/mL were transferred into 10 mL volumetric flasks. The procedure described under "Construction of the calibration curve" was performed. The nominal content of the tablets was calculated using the calibration graph or the corresponding regression equation.

Procedures for stability study

Acidic degradation. Aliquots of GFX standard stock solution (equivalent to 1000 μg of the drug) was transferred into a small conical flask; 5 mL aliquots of 0.1 mol/L HCl was added and heated at reflux for 2 h. At the specified time, the contents of the flask were cooled and neutralized to pH 7.5 with 0.1 mol/L NaOH. The solution was then quantitatively transferred into a 100 mL volumetric flask and completed to volume with distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Alkaline degradation. The alkaline treatment was applied by mixing aliquots of GFX standard stock solution (equivalent to 1000 μg of the drug) with 0.1 mol/L NaOH (5 mL). This mixture was kept at room temperature for 1 h, and neutralized to pH 7.5 with 0.1 mol/L HCl. The solution was then quantitatively transferred into a 100 mL volumetric flask and completed to volume with distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Degradation under neutral conditions. One milliliter of a standard stock solution (equivalent to 1000 μg of the drug) was transferred into a 100 mL volumetric flask; 5 mL of double-distilled water was added and heated using water bath for 3 h at 80 °C. The contents of the volumetric flask were cooled; and completed to volume with distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Oxidative degradation. Aliquots of GFX standard stock solution (equivalent to 1000 μg of the drug) was transferred into a small conical flask; 5 mL aliquots of (3% v/v) H_2O_2 was added and heated at reflux for a period of 2 h. The solution was then quantitatively transferred into a 100 mL volumetric flask and completed to volume with distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Photo degradation. Photolytic degradation studies were also carried out by drug exposure to sunlight and ultraviolet light (254 nm) for a period of 8 h and 3 h, respectively. A stock standard solution of GFX was prepared by dissolving 10 mg of the drug in 10 mL of distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Dry heat studies. To study dry heat degradation, solid drug was exposed in oven at 80 °C for 12 h. After heating, 100 μg GFX was weighed and transferred to volumetric flask (10 mL) and diluted up to the mark with distilled water. One milliliter of the resulting solution was then transferred into a 100 mL volumetric flask and the procedure described under "Construction of calibration graph" was performed.

Results and discussion

Fluorescence spectra

Strong fluorescence was observed for GFX in isopropanol as a solvent at 400 nm after excitation at 272 nm (Fig. 2).

Optimization of experimental parameters

Effect of different diluting solvents. Dilution with different solvents such as methanol, ethanol, dimethyl sulfate, *N*,*N*-dimethylformamide, acetonitrile, water, borate buffer (pH 8), 0.1 mol/L sulfuric acid, 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide was studied. The highest fluorescence intensity was achieved upon diluting with isopropanol. The results are shown in Fig. 3.

Method validation. The validity of the proposed method was tested using the following criterion: linearity, limit of detection,

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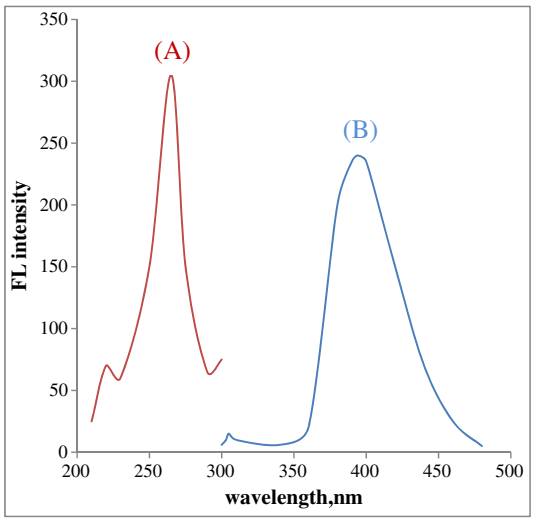

Figure 2. Fluorescence (FL) spectra of (A,B) gemifloxacin mesylate (0.2 μg/mL) in isopropanol. Where (A) is excitation spectra and (B) is the emission spectra.

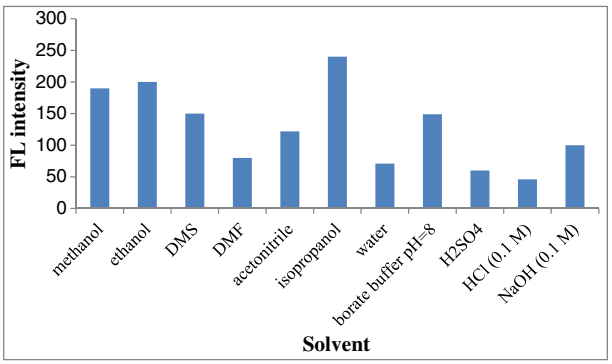

Figure 3. Effect of type of solvent on the fluorescence (FL) of gemifloxacin mesylate (0.2 μg/mL). DMF, *N,N*-dimethylformamide; DMS, dimethyl sulfate.

limit of quantitation, accuracy and precision according to ICH recommendations (26).

Linearity. Assessment of linearity of the assay method was performed by analyzing five sets (standard calibration curve). Adopting the previous procedure, a linear regression equation was obtained. The regression plot showed a linear dependence of GFX on drug concentration over the range of 0.01–0.50 μg/mL. Table 1 shows the results from statistical analysis of data.

Limit of quantification and limit of detection. The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendations (26) below which the calibration graph is nonlinear. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected. The results are summarized in Table 1.

LOQ and LOD were calculated according to the following equations:

Table 1. Performance data for the proposed spectrofluorimetric method for determination of gemifloxacin

Results	Parameter
0.01–0.5	Concentration range, μg/mL
89.29	Intercept (*a*)
761.73	Slope (*b*)
0.9999	Correlation coefficient (*r*)
1.19×10^{-3}	Limit of detection (LOD), μg/mL
3.6×10^{-3}	Limit of quantification (LOQ), μg/mL
0.274	SD of the intercept (S_a)
0.982	SD of the slope (S_b)

$$LOQ = 10S_a/_b$$

$$LOD = 3.3S_a/_b$$

Where, S_a is the SD of the intercept of regression line and *b* is the slope of the regression line.

Accuracy and precision

Statistical analysis (27) of the results obtained by the proposed and reported methods (20) using Student's *t*-test and variance ratio *F*-test showed no significant differences between the two methods regarding accuracy and precision, respectively (Table 2). The intraday precision was evaluated by determination of three concentrations of the GFX drug in pure form on three successive occasions. The interday precision was also evaluated through replicate analysis of three concentrations for a period of three successive days. The results of intraday and interday precision are summarized in Table 3.

Results of stability studies

A variety of stress conditions were trialed. The results of the stress study are presented in Table 4. GFX was stable under dry heat and photolytic degradation conditions and significant degradation of GFX was observed in acidic conditions. Boiling for 2 h with 0.1 mol/L HCl resulted in 81.6% degradation of the original sample. The rate of degradation in alkaline, neutral and oxidative medium is less than degradation in acid medium. The results were comparable to those obtained by the HPLC method. From an economic point of view, the proposed method is simple, rapid and inexpensive, and thus seems a good alternative to previously reported HPLC stability indicating method.

Conclusions

The proposed method was simple, precise and accurate for the determination of GFX. In comparison to previously reported fluorimetric methods, the sensitivity and linear dynamic range were enhanced more. The method was applied for the determination of GFX in dosage forms. Statistical data represent the suitability and reproducibility of the proposed method for routine analysis in quality control laboratories. Because it is rapid and simple, the method has been

Table 2. Application of the proposed and comparison methods to the determination of gemifloxacin in pure form and its pharmaceutical dosage form

	Proposed method			Reported method (20)		
	Taken (μg/mL)	Found(μg/mL)	% Recovery	Taken (μg/mL)	Found(μg/mL)	% Recovery
Pure form	0.05	0.0500	100.00	30	2.9400	98.00
	0.08	0.0790	98.75	3.5	3.4900	99.94
	0.15	1.4900	99.33	4.0	3.9900	99.75
	0.25	0.2480	99.20	4.5	4.4900	99.91
	0.30	0.2980	99.33	5.0	4.9800	99.60
	0.45	0.4490	99.78	5.5	5.4900	99.82
Mean ± SD	99.39 ± 0.442			99.50 ± 0.747		
n	6			6		
Variation ratio	0.195			0.558		
%RSD	0.445			0.751		
t-test	0.311 (2.228)[a]					
F-test	2.86 (5.05)[a]					
Factive® 320 mg/tablet						
	0.05	0.0499	99.70	3.0	2.9800	99.30
	0.08	0.0795	99.40	3.5	3.4900	99.70
	0.15	1.4820	98.80	4.0	3.9500	98.80
	0.25	0.2510	100.40	4.5	4.4600	99.10
	0.30	0.2990	99.90	5.0	4.9800	99.60
	0.45	0.4490	99.70	5.5	5.4900	99.80
Mean ± SD	99.65 ± 0.532			99.38 ± 0.387		
n	6			6		
Variation ratio	0.283			0.1497		
%RSD	0.534			0.389		
t-test	1.006 (2.228)[a]					
F-test	1.890 (5.05)[a]					

RSD, relative standard deviation.
[a]Figures in parentheses are the tabulated *t* and *F* values at confidence limits 95% (27).

Table 3. Validation of the proposed method for the determination of gemifloxacin in pure form

	Found (μg/mL)	% Recovery	% RSD	% Error
Intraday precision				
0.08	0.0796	99.47 ± 0.35	0.35	0.20
0.25	0.2490	99.47 ± 0.61	0.61	0.35
0.45	0.4480	99.57 ± 0.25	0.25	0.15
Interday precision				
0.08	0.0792	99.07 ± 0.31	0.31	0.18
0.25	0.2480	99.20 ± 0.40	0.40	0.23
0.45	0.4460	99.07 ± 0.25	0.25	0.14

RSD, relative standard deviation.

Table 4. Forced degradation study results

Condition	Time (h)	GFX[a] (%)
Acid (0.1 mol/L HCl, heated at reflux)	2	18.4
Base (0.1 mol/L NaOH, room temperature)	1	66.6
Water (80 °C)	3	84.7
Hydrogen peroxide (3%, heated at reflux)	2	62.8
Heat dry, 80 °C (solid)	12	99.9
Exposure to sunlight	8	98.6
Exposure to ultraviolet light (254 nm)	3	99.6

GFX, gemifloxacin.
[a]Remaining GFX (%) after exposure to stress conditions.

adapted for stability studies of the drug as an alternative for the reported stability-indicating HPLC method.

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